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13. ABSTRACT (Maximum 200 Words)

This project is designed to test the hypothesis that chronic exposure to depleted uranium (DU) impairs neuronal processes underlying cognitive function via alterations induced at hippocampal glutamatergic synapses. As prescribed by the Statement of Work, efforts in year 2 concerned completion of Technical Objective 1 (establishment of chronic exposure protocol) and achieving substantial progress on Technical Objective 2 (defining integrity of hippocampal glutamate release). Blood and brain concentrations of uranium (U) increased monotonically as a function of exposure level and duration up to 12 months, and were correlated with a decrease in rate of growth. Acute exposure to U *in vitro* diminished K^+-stimulated glutamate and GABA release in a concentration-dependent fashion in hippocampal synaptosomes. The inhibitory effect on evoked glutamate release was more potent (IC_{50} = 2.61 μM) than that on GABA (IC_{50} = 204 μM), and was similar to IC_{50} values for transmitter release exhibited by other multivalent metal ions. Studies are currently underway examining the effects of chronic exposure on transmitter release *in vivo*. Given the similarity of effects of U on transmitter release to those of other multivalent metals and the fact that exposure in military scenarios is continuing, additional studies are warranted on uranium actions in several experimental settings.

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Depleted uranium, glutamate release, military disease, NMDA receptor, AMPA receptor, hippocampus, microdialysis

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Introduction

The chemical properties of depleted uranium (DU) render the metal well suited for military purposes. The U. S. Army utilizes DU for tank armor and for kinetic energy penetrators in munitions, and has deployed such weapons in the Gulf War, in Kosovo, and in Iraq. Use of the metal in future military arenas is a virtual certainty, but knowledge of its toxicity is lacking. Gulf War veterans who retained fragments of DU shrapnel over several years have exhibited lowered performance on neurocognitive tests (1). Moreover, research in chronically exposed rats has indicated alterations in hippocampal synaptic transmission, suggesting DU-induced decreases in neuronal excitability (2). *This research proposal will therefore test the overall hypothesis that chronic exposure to DU impairs neuronal processes underlying cognitive function via alterations induced at hippocampal glutamatergic synapses that directly modulate* Ca^{+2}*-mediated cellular processes.* Glutamatergic function will be assessed in rats exposed for 12 months via intramuscular implants of varying amounts of DU pellets in order to identify the bases for the impaired cognition and diminished neuronal excitability. Components of depolarization-evoked glutamate release will be measured in the presence of acute *in vitro* or after extended *in vivo* exposure to the metal (Technical Objective 2). Determination of the actions of uranium on glutamatergic NMDA and AMPA receptors will be performed via approaches employing analogous *in vitro* and *in vivo* exposures (Technical Objective 3). Other studies will determine the concentrations of DU produced in blood and brain tissue as a result of exposure (Technical Objective 1). These results will be of critical importance to U. S. armed forces in defining risk and establishing treatment modalities for DU exposures sustained in recent conflicts and in future battlefield situations.

Body

As prescribed in the approved Statement of Work, project activities in year 2 addressed Technical Objectives 1 and 2. A description of these efforts and the progress toward completion of each Objective is provided below.

Technical Objective 1 concerned establishment of the chronic DU exposure protocol, and this work has been completed. Rats were exposed to 0 (controls), 300, or 600 mg DU by implantation of 30-mg (2 mm × 1 mm diameter cylinders) pellets in the gastrocnemius muscles of their hindlimbs. Tantalum pellets of the same size were used in control animals and to balance the total metal mass implanted across groups with an inert metal. Male Sprague-Dawley rats, 70-100 days of age, were anesthetized with ketamine (87 mg/kg)/xylazine (13 mg/kg). The abdomen was shaved and the surgical site prepped with povidone-iodine and isopropyl alcohol. Each foot was wrapped in sterile gauze and the leg surrounded by a sterile drape. A 2.0 cm incision was made in the leg until the gastrocnemius muscle was visualized. Pellet implantation began by inserting the tip of an 18-gauge needle into the incision. A 16-gauge Rosenthal needle (J.A. Webster, Inc.) was then positioned 2 mm down into the muscle and the pellet inserted via the needle plunger. The remaining 9 pellets were inserted in the same manner approximately 1.0 mm apart. The incision was closed using 4.0 Ethilon PS-2 suture utilizing a running stitch. A triple antibiotic ointment was applied to the wound, an injection of ampicillin (100,000 IU/kg) was given intramuscularly, and the rat placed under heat until awakening. An Elizabethan collar was utilized for 24 hours to allow the wound to heal. Animals were anesthetized again with

ketamine/xylazine after exposure periods of 1, 3, 6, or 12 months (N = 6/group/time period), and 1 ml of blood collected by cardiac puncture and placed into heparinized tubes. The rats were then sacrificed and hippocampus dissected from whole brain tissue and both tissues weighed and stored at -20°C. Body weights of all animals were recorded weekly during exposure.

DU pellets (0.75% tungsten, 99.25% uranium) were obtained from Manufacturing Sciences Corp. (Oak Ridge, TN) or Aerojet Ordnance (Jonesborough, TN), while tantalum wire was obtained from Aldrich (St. Louis, MO). Prior to implantation pellets were chemically cleaned by brief soaking in 50% nitric acid, rinsing with distilled water, and drying in a stream of argon. Pellets were stored under argon until use, and briefly immersed in isopropyl alcohol just prior to placement.

To comply with uranium disposal regulations pellets were recovered from all animals after sacrifice. An incision is made in each leg over scar tissue from the previous implantation, and cuts are made through single layers of muscle until pellets are visualized and removed using forceps. Utilizing these procedures pellet recovery is virtually 100%. Degradation of the uranium pellets is readily observed, while the degree of oxidation increases with length of implantation.

For elemental analysis portions of blood samples (approximately 600-1000 µl) and hippocampi from each animal were weighed into Teflon digestion vessels, and nitric acid and hydrogen peroxide (0.5-1.0 ml) added. The digestion vessels were sealed, heated in a microwave digestion system, and cooled to room temperature. The resulting digestates were transferred to preweighed metal-free polypropylene centrifuge tubes. Samples were weighed to calculate exact mass dilution factors. A solution of thorium internal standard was added to compensate for matrix effects during analysis and the vessels reweighed. Additionally, a depleted uranium pellet was weighed into a metal-free polypropylene tube and dissolved at room temperature with nitric acid. This solution was diluted to approximately 20 ppb for isotope ratio measurements. Vessel blanks, containing no sample but otherwise heated and processed in the same way as the samples, were prepared with every digestion set in order to assess vessel and reagent backgrounds.

Analyses were performed by Elemental Analysis Inc. (Lexington, KY) with a VG PlasmaQuad 3 Quadrapole ICP-MS calibrated using five linearity standards prepared from dilutions of a single-element U standard. The isotope ratios of this standard were similar to those of the depleted uranium pellet. Only very small amounts of uranium were found in vessel blanks, and backgrounds have been subtracted from the results where appropriate. Quality control (QC) standards and blanks were analyzed repeatedly through the analytical runs to ensure instrument stability and to monitor background signals.

The results of the determinations of uranium in whole blood samples are summarized in Figure 1. Uranium concentrations in chronically exposed animals increase monotonically up to 12 months duration in an exposure level-dependent fashion. Values in the high dose group reach ~3.0 ng/ml after 12 months' exposure. Urinary uranium levels reported by Pellmar *et al.* (3) utilizing a similar exposure protocol are maximal at 12 months duration, but it is not clear whether blood or serum values follow a similar pattern.

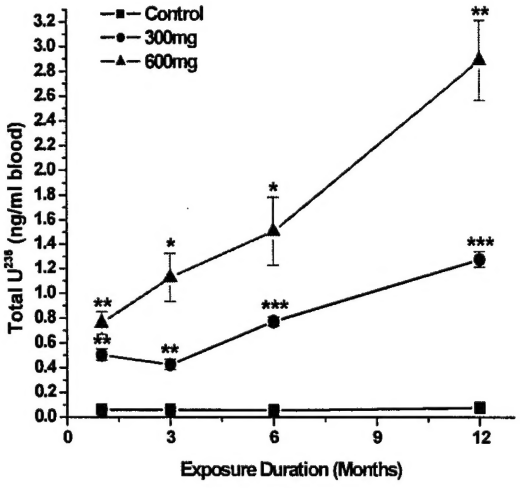

Figure 1. Concentrations of uranium in rat whole blood as a function of amount and duration of exposure to implanted DU pellets as determined by ICP-MS. Control and low dose (300 mg) DU groups received tantalum pellet implants so that all animals received the same total mass of metal. Values are expressed as mean ± SEM with N = 6 for each exposure group and duration. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ compared to control values at the same exposure duration.

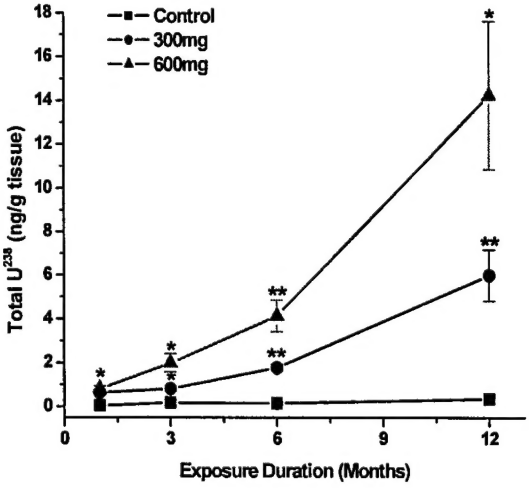

Figure 2. Concentrations of uranium in rat hippocampus as a function of amount and duration of exposure to implanted DU pellets as determined by ICP-MS. Control and low dose (300 mg) DU groups received tantalum pellet implants so that all animals received the same total mass of metal. Values are expressed as mean ± SEM with N = 5-6 for each exposed group and duration. Control values were not changed as a function of duration and thus were combined into a single group (N = 13). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ compared to control values.

Analogous values for hippocampal uranium values are shown in Figure 2. Similar to the blood levels, uranium concentrations in chronically exposed animals increase monotonically up to 12 months duration in an exposure level-dependent fashion. Values in the high dose group reach ~14 ng/g tissue after 12 months exposure, and are substantially lower than those reported elsewhere with similar uranium exposure protocols (3-4). Postmortem tissue weights for hippocampus and the remaining brain did not differ as a function of uranium exposure (data not shown).

Somatic growth for each group as a function of exposure duration is summarized in Figure 3. An analysis of variance uncovered a significant effect of exposure ($p < 0.016$), manifested as a diminished rate of growth in the high dose DU group (Scheffe *post hoc* test, $p = 0.023$). This effect also approached statistical significance in the lower dose DU group ($p = 0.079$). The impaired rate of growth observed in this work is remarkably similar to that reported by Pellmar *et al.* (4) who used a similar exposure regimen. The agreement in these findings indicates the reproducible nature of the effect of DU exposure on growth. During the course of these studies one control animal developed an ear infection and was removed from the study. In addition, two low dose and three high dose DU animals either developed tumors or died between 6 and 12 months of exposure.

As a result of this work DU pellet implant procedures have been standardized, resulting in a virtually complete recovery of pellets after animal sacrifice. Furthermore, the exposure protocol has been established with blood and brain uranium concentrations in conjunction with body

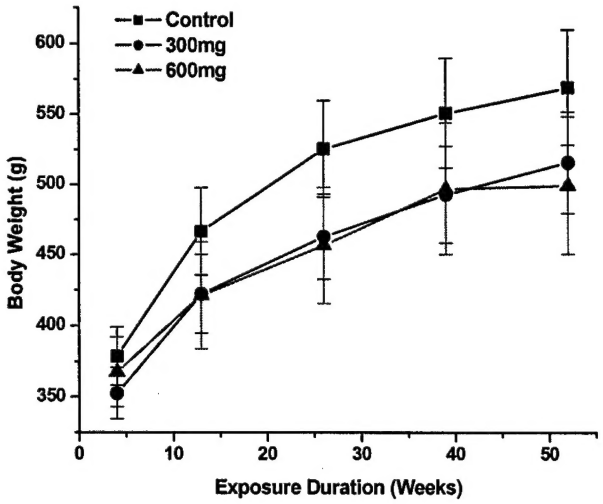

Figure 3. Increases in whole rat body weight as a function of amount and duration of exposure to implanted DU pellets. Control and low dose (300 mg) DU groups received tantalum pellet implants so that all animals received the same total mass of metal. Data were evaluated by an analysis of variance comparing cumulative weight gain across exposure groups using pre-implantation body weight as a starting point. This resulted in a significant effect of exposure ($p = 0.016$). Values are expressed as mean $\pm$ SD with N = 5-6 for each exposed group and duration. An overall significant decrease in body weight was uncovered in the high DU group (Scheffe *post hoc* test, $p < 0.05$).

weight curves reflecting diminished growth. It is also noteworthy that the hippocampal uranium levels measured by ICP-MS are much lower than those reported using kinetic phosphorescence (3-4), even in the presence of similar decreases in body weight.

There were no significant problems encountered in the accomplishment of Technical Objective 1. A few hippocampal samples from control animals at 6 or 12 months after implant surgery were found to be obviously contaminated with uranium and were removed from the sample set. An additional batch of DU pellets were ordered from a different vendor early in year 2 – this represented a substantial cost savings, but quality of the product was not acceptable. These concerns were subsequently resolved by replacement of the defective pellets.

Technical Objective 2 concerned definition of the integrity of hippocampal glutamate release as a result of acute or chronic uranium exposure. Substantial progress has been made in the acute exposure studies utilizing a superfusion procedure that permitted measurement of endogenous glutamate and GABA release from hippocampal synaptosomes. A range of uranium concentrations was applied to the synaptosomes via the superfusing buffer to permit estimation of the inhibitory/stimulatory potency on the transmitter release component under study. Hippocampi from eight 60-70 day old male Sprague Dawley rats were collected and homogenized in a glass vessel with 0.2-0.25mm total clearance (Kimble Kontes, Vineland, NJ) containing 10 mM HEPES-0.32M sucrose buffer (pH 7.4). The homogenate was centrifuged for 2 min at $3000 \times g_{max}$, and the supernatant removed and transferred to a clean tube. This supernatant was centrifuged again for 12 min at $14{,}600 \times g_{max}$, resulting in isolation of a synaptosomal pellet. The pellet was resuspended in HEPES-sucrose buffer and stored on ice for 30 minutes until protein concentration was determined using the bicinchoninic acid assay (5). Synaptosomes were then diluted to 1.0 mg protein/ml with an isotonic HEPES buffer (containing in mM: NaCl 132, KCl 1, $MgCl_2$ 1, $CaCl_2$ 0.1, glucose 10, HEPES 10, and 0.1% BSA; bubbled in 99.9% O_2, pH 7.40) and incubated for 30 min at 37°C.

The synaptosomes were centrifuged for 30 sec at $15{,}800 \times g_{max}$ and the supernatant removed prior to resuspension to 4.0 mg/ml in the above HEPES-buffer (without BSA, and containing a

glutamate reuptake blocker - 0.5 mM DL-threo-β-hydroxyaspartic acid). Exposure solutions also contained uranium oxynitrate (Noah Technologies, San Antonio, TX) or uranium (VI) oxide (Ultra Scientific, North Kingstown, RI) at concentrations ranging from 10^{-3} to 10^{-8} M. 200µl of synaptosomes were then pipetted into each chamber (Model SF-12, Brandel, Gaithersburg, MD), and superfusion initiated with the same HEPES buffer at 0.6 ml/min. Flow was maintained for 30 min and then baseline samples were collected at 2 min intervals. During perfusate collection superfusion was switched for 2 min to the same HEPES buffer containing 31 mM KCl (Na^+ reduced to maintain isotonicity) and then returned to the normal buffer to re-establish the baseline.

After superfusion was complete, each sample was prepared for HPLC analysis by derivatization using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. (AccQ-Tag, Waters Corp., Milford, MA). The derivatives were then analyzed using binary gradient liquid chromatography with fluorescence detection (excitation - 250 nm and emission - 395 nm). Eluent A consisted of Na acetate, pH 5.70 containing triethylamine, while eluent B was a 60:40 mixture of acetonitrile:water. Detector output was analyzed by EZChrom Elite software (Scientific Software, Pleasanton, CA).

While it is more common to measure synaptosomal glutamate/GABA release employing ^3H-amino acids that are loaded into synaptosomes and then released by high K^+ stimulation, it is generally accepted that this form of release may not be drawn from the same intracellular pools as release of the endogenous transmitter. However, endogenous release is more analytically difficult to detect because of the small concentrations involved. The superfusion-liquid chromatography system described above possesses sufficient sensitivity to measure these latter concentrations.

The presence of uranium (VI) oxide or uranium oxynitrate (i.e., uranyl ion, UO_2^{+2}) in the superfusion buffers clearly diminished depolarization-evoked synaptosomal glutamate and GABA release across the range of metal concentrations tested. Figure 4 shows the time course of the effects of two concentrations of uranium compared to glutamate release from a control preparation. The high K^+ stimulus elicited a maximal response that represented an approximate 3-fold increase in non-exposed synaptosomes, while smaller increases (if any) were seen in the presence of superfused uranium. A similar effect on the time course of K^+-stimulated hippocampal GABA release is shown in Figure 5. Again, the maximal response is an approximate 3-fold increase in release with smaller responses observed in the presence of concentrations of uranium.

If the area under the stimulation response curves is computed and compared across uranium concentrations to the control area, a percent inhibition of the response can be calculated for each level of uranium. These transformations result in the inhibition curves for stimulated glutamate and GABA release shown in Figure 6. The effect of acute exposure to the metal is markedly more potent with respect to glutamate release (IC_{50} = 2.61 µM) than it is for GABA release (IC_{50} = 204 µM).

The basis for the differential potency of uranium on glutamate and GABA release is not known. It is possible that uranium binds glutamate (6) – but not GABA – in a soluble complex, but an

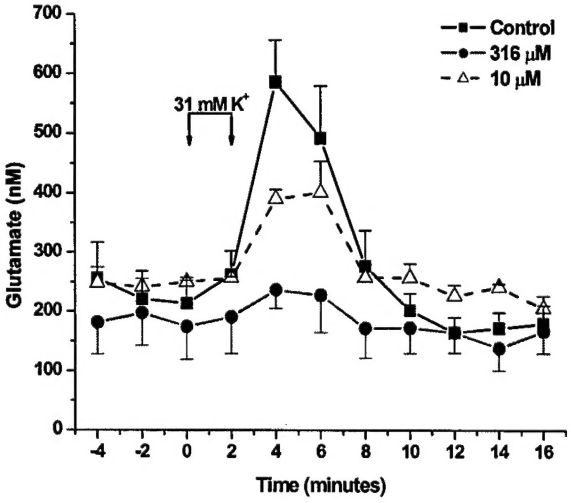

Figure 4. Time course of glutamate concentration in response to superfusion with 31 mM K^+ across hippocampal synaptosomes in standard 10 mM HEPES-sucrose buffer (pH 7.4) or in buffer containing one of two concentrations of uranium. The stimulation-evoked increase in endogenous glutamate was diminished by the presence of uranium. Values are expressed as mean ± SEM of independent determinations – N = 5 for control conditions, and 2 for each uranium concentration) – conducted in triplicate.

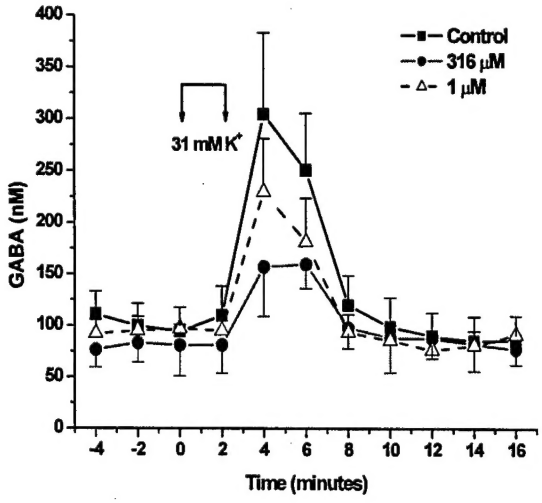

Figure 5. Time course of GABA concentration in response to superfusion with 31 mM K^+ across hippocampal synaptosomes in standard 10 mM HEPES-sucrose buffer (pH 7.4) or in buffer containing one of two concentrations of uranium. The stimulation-evoked increase in endogenous GABA was diminished by the presence of uranium. Values are expressed as mean ± SEM of independent determinations – N = 5 for control conditions, and 3 for each uranium concentration) – conducted in triplicate.

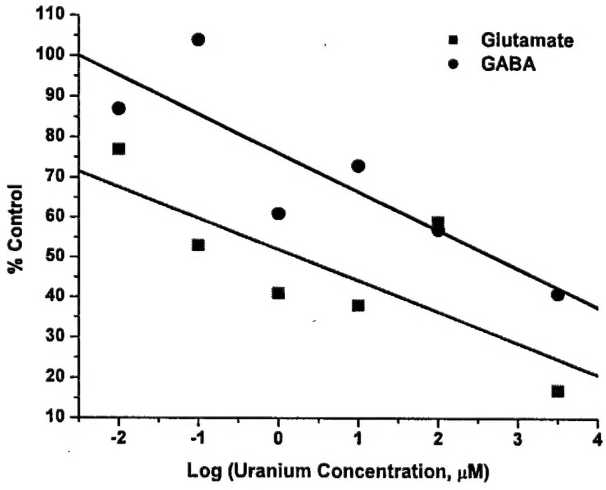

Figure 6. Percentage inhibition of stimulated glutamate and GABA release from hippocampal synaptosomes as a function of the concentration of uranium in the superfusing solution. Glutamate release is more sensitive to acute uranium exposure than that of GABA. Maximal inhibition is limited by the solubility of uranium in aqueous media at pH 7.4. The data were fitted by linear regression of the types of response inhibition shown in Figures 4 and 5.

effect that would diminish the derivatization efficiency for glutamate and account for the differential potency has not been observed. Alternatively, it is plausible that uranium could complex glutamate in such a fashion as not to affect derivatization but that might more potently bind to synaptic sites related to transmitter release, e.g., to block voltage-sensitive Ca^{+2} channels. Elucidation of the mechanism(s) involved will require further investigation.

However, the uranium IC_{50} for glutamate release of 2.61 μM based on nominal metal concentrations is remarkably similar to the inhibitory potencies of a number of other multivalent metal ions (7). This suggests by inference that the action of uranium is exerted at membrane voltage-sensitive Ca^{+2} channels to interfere with Ca^{+2} influx and diminish exocytosis. Moreover, some of these multivalent metals (e.g., lead) are well known to possess developmental neurotoxicant properties. Thus, it is clear that additional studies are warranted on uranium actions in an array of experimental settings.

The uranium species involved in the effect on exocytosis is not known. This study has utilized both uranium (VI) oxide and uranyl nitrate, but data analysis has not been completed on sufficient experimental replications at this time to distinguish the actions of these two salts. Uranyl ion (UO_2^{+2}) – the most common form produced in the body from all forms of the metal – is also converted to diuranate ion ($U_2O_7^{-2}$) under alkaline conditions (8), and this uranium complex may also be involved.

Completion of the acute *in vitro* exposure studies also will involve an assessment of the Ca^{+2}-mimetic properties of uranium by measuring depolarization-evoked synaptosomal release in the absence of Ca^{+2} in the superfusion buffer. Preliminary studies have shown that this component of glutamate release constitutes only ~25-30% of the total release observed under the conditions described above. If uranium at least partially supports exocytosis – like Pb^{+2} – then this release component should be greater than that found in control preparations. Alternatively, a more direct determination of this effect can be obtained by permeabilizing the synaptosomes with detergent so that the uranium species cannot enter the cell by diffusion and exert its action.

Additional studies examining total and Ca^{+2}-independent release will utilize Pb^{+2} as a positive control as it has well-established acute effects *in vitro* on voltage-sensitive Ca^{+2} channels as well as Ca^{+2}-mimetic properties. Experiments will also be conducted utilizing chelating agents and free uranium ion concentrations to more precisely and reliably identify its potency for glutamate and GABA release.

The accomplishment of Technical Objective 2 will be completed with analogous experiments performed *in vivo* employing intracerebral microdialysis to quantify the changes in hippocampal glutamate/GABA release occurring after a chronic exposure period of 12 months. All animals for this study have been implanted and chronically exposed (N = 15-16/exposure group) and the microdialysis sessions have begun.

Despite the above project accomplishments, two matters developed in year 2 that hindered further progress. The recruitment of a Chinese postdoctoral person for the project eventually failed because of the protracted visa approval process and the candidate's unwillingness to remain available. Shortly thereafter, a senior research technician with several years experience and an M.D. from the People's Republic of China became available locally and was hired on the project. As a result, the absence of the postdoctoral person will not have a significant long-term effect on achievement of research aims.

The dearth of information in the literature on uranium chemistry and the metal's solubility in physiological buffers posed transient challenges. Uranium is poorly soluble at alkaline pH,

precipitates phosphate, and forms complexes with carbonate ion. The composition of superfusion buffers thus had to be modified. Also, the higher concentrations of uranium utilized in the superfusion experiments have an adverse effect on chromatographic performance, resulting in split or asymmetric peaks. We tested approaches to treat the superfusion samples to remove/inactivate uranium prior to derivatization and chromatography, but found no solutions that achieved this goal without causing other equally significant problems. We have concluded that the most efficient response to the problem is to simply accept a faster rate of guard and analytical column aging.

Key Research Accomplishments

The key research accomplishments on this project to date are:

- Standardization of DU/tantalum pellet preparation and implantation procedures
- Establishment of chronic DU exposure protocol with blood and brain uranium concentrations
- Assessment of chronic DU exposure on rate of growth
- Demonstration that acute uranium exposure *in vitro* diminishes endogenous depolarization-evoked synaptosomal glutamate and GABA release
- Identifying the potency of uranium to diminish stimulated glutamate release as similar to that of other multivalent metal ions, thereby opening other avenues of investigation

Reportable Outcomes

Lasley, S.M. and Vietti, K.R. Acute exposure to uranium decreases potassium-stimulated hippocampal glutamate release. The Toxicologist, 78, 1140, 2004.

Lasley, S.M. and Vietti, K.R.N. Acute exposure to uranium *in vitro* decreases potassium-stimulated hippocampal glutamate release. Military Health Research Forum, San Juan, Puerto Rico, April 25-28, 2004.

Lasley, S.M. and Vietti, K.R. Chronic exposure indices resulting from implanted depleted uranium pellets. In preparation.

Lasley, S.M. and Vietti, K.R. Acute exposure to uranium diminishes potassium-stimulated glutamate and GABA release in hippocampal synaptosomes. In preparation.

Conclusions

Blood and hippocampal uranium concentrations in chronically exposed animals increase monotonically up to 12 months exposure duration. In addition, rate of somatic growth is significantly impaired in the high dose DU group compared to controls. However, the

hippocampal uranium levels measured by ICP-MS are substantially lower than those reported by Pellmar *et al.* (3-4) even though the observed decrease in body weight (4) is remarkably similar to that reported in Figure 3.

Acute uranium exposure *in vitro* clearly produces an inhibitory effect on potassium-stimulated Ca^{+2}-dependent glutamate and GABA release in hippocampal synaptosomes, exhibiting a more potent effect on the glutamatergic process. The IC_{50} (2.61 uM) for glutamate release based on nominal uranium concentrations is remarkably similar to those determined for other multivalent metal ion effects on transmitter release.

The value of this knowledge as a scientific product resides in the establishment of the DU chronic exposure protocol as a shrapnel wound model based on blood and brain levels of the metal and rates of growth. These measures provide benchmark values for future studies in this project and for correlation of results from this project to those obtained in other laboratories. In addition, given the similarity of the effects of uranium on transmitter release to those of other multivalent metals (e.g., lead) and the fact that exposure in military scenarios is continuing, it is clear that additional studies are warranted on uranium actions in an array of experimental settings. It is particularly noteworthy that some of the multivalent metals exhibiting effects on transmitter release (e.g., lead) possess well-known developmental neurotoxicant properties.

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Appendices

The two published abstracts cited above are attached.

ACUTE EXPOSURE TO URANIUM IN VITRO DECREASES K^+-STIMULATED HIPPOCAMPAL GLUTAMATE RELEASE

Lasley SM and Vietti KR

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BACKGROUND/PURPOSE: Gulf War veterans who retained fragments of depleted uranium (DU) shrapnel have exhibited lowered performance on neurocognitive tests, and rats chronically exposed to DU have displayed apparent decreases in hippocampal neuronal excitability. The goal of this study was to determine if acute exposure to uranium (U) in vitro disrupts synaptic processes underlying K^+-stimulated neurotransmitter release.

METHODS: A crude hippocampal P_2 fraction was prepared from 2-3-month old Sprague-Dawley rats, and 0.8 mg synaptosomal protein loaded onto glass fiber filters. Synaptosomes were superfused with a phosphate-free 10 mM HEPES buffer saturated with O_2 and maintained at pH 7.4. Endogenous release was stimulated with brief perfusion of high K^+-HEPES containing a glutamate reuptake blocker. Concentrations of U oxide (U(VI), 0.1 uM – 1.0 mM) were added to perfusion solutions, and 2-minute fractions were collected beginning ~45 minutes after exposure was initiated. Aliquots were derivatized and quantified by binary gradient liquid chromatography with fluorescence detection.

RESULTS: In the absence of U, K^+ stimulation resulted in a four- to sixfold elevation in glutamate concentration over baseline values. Perfusion of U(VI) diminished stimulated endogenous K^+-evoked glutamate release at 1.0 uM (-48%) and 1.0 mM (-80%) compared to control values, resulting in an approximate IC_{50} of 4.25 uM.

CONCLUSION: The duration of U(VI) exposure is appropriate for investigating its effects on synaptosomal membrane calcium channels involved in exocytosis, but this form of the metal is somewhat less potent in this respect than other more well-studied cations such as divalent lead or cadmium. Other more biologically significant forms of U (e.g., uranyl ion, UO_2^{+2}) may exhibit greater potency on transmitter release processes. The intracellular actions of U(VI) on exocytosis could also be important. These initial findings suggest a significant effect of U(VI) on hippocampal glutamatergic transmission and indicate the need for further investigation.

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ACUTE EXPOSURE TO URANIUM (U) DECREASES POTASSIUM – STIMULATED HIPPOCAMPAL GLUTAMATE

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Gulf War veterans who retained fragments of depleted uranium (DU) shrapnel have exhibited lowered performance on neurocognitive tests, and rats chronically exposed to DU have displayed apparent decreases in neuronal excitability. The goal of this study was to determine if acute exposure to U in vitro disrupts synaptic processes underlying K+-stimulated glutamate release. A crude hippocampal P2 fraction was prepared from 1-2 month old Sprague-Dawley rats, and 0.8 mg synaptosomal protein loaded onto glass fiber filters. Synaptosomes were superfused with a phosphate-free 25 mM HEPES buffer saturated with 02/CO2 (95:5) and maintained at pH 7.4. Release was stimulated with brief perfusion of high K+-HEPES containing a glutamate reuptake blocker. Concentrations of U oxide (U(V), 0.1 uM to 1.0 mM) were added to perfusion solutions, and 2-min fractions were collected beginning ~45 min after exposure was initiated. Aliquots were derivatized and quantified by binary gradient liquid chromatography and fluorescence detection. In the absence of U, K+ stimulation resulted in a 2-4-fold elevation of glutamate concentration over baseline values. Perfusion of U(VI) diminished endogenous potassium-evoked glutamate release at 0.1 mM (-37%) and 1.0 mM
(-70%) compared to control values, resulting in an approximate IC50 of 0.25 mM. The duration of U(VI) exposure is appropriate for investigating its effects on membrane calcium channels involved in exocytosis, but this form of the metal is less potent than other more well studied divalent cations such as Pb+2 or Cd+2. Other more biologically significant forms of U (e.g. uranyl ion, divalent U02) may exhibit greater potency on transmitter release processes. The intracellular actions of U(VI) on exocytosis could also be important. These initial findings suggest a measurable effect of U(VI) on hippocampal glutamatergic transmission and indicate the need for further investigation. (Supported by USAMRMC grant DAMD17-02-1-0212)

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